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Original Article

Transcranial magnetic stimulation in sleep fragmentation: a model to better understand sleep disorders

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ABSTRACT

Objective: To investigate practice-dependent plasticity and cortical inhibition/excitability in good sleepers after a night of sleep fragmentation (SF), by means of transcranial magnetic stimulation (TMS).
Methods: In basal condition (BC), after a full night of spontaneous sleep, and in fragmented condition (FC), after a fragmented night of sleep, motor evoked potential (MEP) amplitude, motor threshold (MT), silent period (SP), and intracortical inhibition were assessed. In both conditions subjects performed, also, a bimanual motor task: MEPs were recorded before and after exercise, and after rest. We evaluated the presence of post-exercise facilitation and delayed facilitation. Subjects reported their alertness level (Stanford Sleepiness Scale–SSS).
Results: MT and SSS were significantly increased in SF. Instead, no significant differences for MEP amplitude or SP or intracortical inhibition were found. In both conditions post-exercise facilitation and delayed facilitation were present.
Conclusion: SF produces disruption of nocturnal sleep and increases daytime sleepiness. Confirmatory features of this clinical behaviour could be that in FC we observed a significant increase in SSS and in MT. SF was unable to modify cortical inhibition\excitability and\or to influence plasticity-related parameters. These results seem inconsistent with some of TMS alterations observed in sleep deprivation (SD) and restless legs syndrome (RLS). We suggest that SD and SF represent different phenomena that can depend on various networks acting on motor cortex. We speculate that alterations in cortical excitability found in RLS are intrinsically related to the underlying disease itself and are not instead directly associated with the SF present in RLS.

1. Introduction

Learning and memory processes depend on brain plasticity defined as structural and/or functional neural changes in response to stimuli [1]. Sleep seems to play a key role in modulating and regulating these processes. Therefore, sleep loss and generally sleep disorders are crucial for sleep-dependent plasticity [2]. From a scientific standpoint, the term “sleep loss” covers a wide range of sleep loss types, which differ in the kinds of electroencephalogram (EEG) findings, sleep macro- and microstructures, clinical presentations, and behavioural and alertness effects. First, sleep diseases that induce sleep loss per se are distinguished from artificial laboratory conditions that simulate the effects of common sleep disorders such as sleep deprivation (SD) (which can be total, partial or selective SD) and sleep fragmentation (SF) [3]. SD is defined as the length of time since the end of the last sleep period [4]. SF is an interruption of the normal continuity of sleep with frequent and transient EEG arousals [5]. Generally, the arousals produce fragmented rather than shortened total sleep time, as in SD [4]. SF instead is a sleep disruption characterized by an altered sleep quality rather than sleep quantity. SF and SD are both associated with an increased sleepiness the following day with the impairment of daytime cognitive performance and neurophysiological functions [4–9]. The alterations in cognitive performance and the instability of the waking state after SD seem to reflect changes in neurotransmitter receptor functionality related to sleep loss. In particular, insufficient sleep may alter neuronal excitability and synaptic communication in neuronal networks implicated in cognition, learning, memory, and brain plasticity [2,10]. SF seems to impair the restorative/cognitive benefits of sleep via alterations in hippocampal synaptic plasticity, but it involves mechanisms different from those altered in SD. Thus, alterations in *N*-methyl-D-aspartate receptors have not yet been shown for SF, which is likely to be the more clinically relevant model for sleep disorders compared to total or selective rapid eye

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movement (REM) SD [11]. Whereas many previous animal studies have investigated the neurophysiological and behavioural effects of sleep loss, much less is known concerning its effects on the human brain. Few previous studies by means of transcranial magnetic stimulation (TMS) technique have examined non-invasively the effects of SD on cortical inhibition/excitability [12–17]. In our previous study, we demonstrated that, in sleep-deprived subjects, a marked decrease of the central motor inhibition is present [14,17]. Modifications in excitatory and inhibitory systems are also present in different sleep disorders. In restless legs syndrome (RLS), a sleep disorder clinically characterized by markedly fragmented sleep, we demonstrated alterations in movement-related cortical plasticity [18–21], which dopaminergic treatment can reverse [20].

To the best of our knowledge, there have been no previous human studies concerning the influence of SF on cortical inhibition/excitability and/or in cortical plasticity-related parameters.

To date, another important question has not yet been addressed: whether the alterations in cortical excitability found in RLS are intrinsically related to the underlying disease itself or whether they depend directly on the RLS-related SF.

2. Methods

2.1. Subjects

Seven young healthy male volunteers (aged 20–25 years), four right-handed and three left-handed, with no history of sleep disorders or any other disease were recruited from the local student population. We enrolled only males to avoid possible influences of sexual hormones on the circadian factors. All participants were required to maintain a regular sleep schedule during the 2 weeks before the study sessions, as verified by a sleep diary. No alcoholic or caffeinated beverages after 16:00 and no napping were allowed on study nights.

According to the experimental design, for each subject the assessment of motor cortex excitability was performed separately in two different conditions: basal condition after a full night of spontaneous sleep at home and fragmented condition after a fragmented night of sleep at the sleep laboratory. The interval between the two sessions was not strictly predefined, but it was longer than 1 week. The order of the basal condition and fragmented condition session was counterbalanced across subjects. The morning following both the basal and the fragmented night, before the experimental session, subjects were asked to report their alertness level on a seven-point scale (Stanford Sleepiness Scale – SSS) in which each successive number represents an increasing level of sleepiness [22]. Prior to taking part in the study, all participants were informed about the nature of the study and provided written consent.

2.2. Fragmentation protocol

Subjects underwent three consecutive night polysomnographies. In our sleep laboratory, each subject was monitored continuously for 9 h (22:00–07:00). During each night, EEG (C4–A1, C3–A2, O2–A1, and O1–A2), electro-oculogram (EOG), submental electromyogram (EMG), tibial anterior EMG of both legs, nasal and oral airflow, movements of the chest and abdomen, snoring, transcutaneous oxygen saturation, and pulse rate were recorded. The first two nights were used for adaptation to the laboratory. On the third night, the sleep of the subjects was fragmented by means of acoustic stimulations delivered through loudspeakers. SF procedures were initiated after 10 consecutive minutes of sleep (stage 2, slow wave sleep) were achieved. Tone generation occurred throughout the entire night in all stages of sleep. The tone generation program was designed to vary tone duration, volume, and frequency in an effort to reduce adaptation. During fragmentation these three parameters were varied in order to produce an abrupt shift in EEG frequency to theta, alpha, and/or frequencies >16 Hz of ≥3 s in duration but ≤15 s on the EEG channels, or to slow EEG features such as K-complexes and transient delta activities. When such response was achieved, the next acoustic stimulation was given after 120 s. If nothing was seen on the EEG, frequency, volume, and duration of tones were changed until the appearance of an arousal. Tone frequency was either 1000 or 2000 Hz. Duration of tones varied between a minimum of 450 ms and a maximum of 8625 ms and their intensity between 50 and 90 dB.

2.3. Transcranial magnetic stimulation

TMS was performed with a MagLite-r25-Twin Top, Medtronic A/S biphasic stimulator (Copenhagen, Denmark) using single- and paired-pulse procedures. The non-dominant hemisphere was stimulated because delayed facilitation in healthy subjects is limited to this hemisphere [23,24] and because an inter-hemispheric asymmetry in the excitability of cortical inhibitory mechanisms has been demonstrated [25]. In both conditions, in order to prevent the effect of circadian factors, the recording sessions were performed always in the late morning. In order to avoid vigilance fluctuations, the subjects under TMS session were asked by the investigator to remain alert with open eyes, but in a relaxed body condition.

TMS was performed according to published protocols [18–20,23,24]. After having verified the integrity of peripheral motor conduction, F-wave, and central motor conduction time, three experimental sessions were performed on each subject for each condition (basal condition and fragmented condition): (1) evaluation of motor evoked potential (MEP) parameters: motor threshold, MEP amplitude, and silent period duration, were measured in response to single-pulse magnetic stimulation [26,27]; (2) motor task: MEPs were recorded in response to single magnetic stimuli after a motor task [18–20,23,24]; (3) paired-pulse stimulation: the time-course of intracortical motor activity was tested using pairs of magnetic stimuli (1–6 ms inter-stimulus intervals) [14,18–20,28].

2.4. Single-pulse TMS: MEP amplitude, motor threshold, and silent period

MEPs were recorded from the first dorsal interosseous muscle of the non-dominant hand via surface electrodes applied in a belly-tendon montage. A round coil (90 mm) was used, and the lateral edge was placed over the presumed hand area. The coil handle was held backward in a lateral (45°) direction from the interhemispheric line. The optimal scalp position was determined by moving the coil in 1 cm steps over the presumed hand motor area. For motor threshold measurement, MEPs were recorded during relaxation of the target muscle. A moderate contraction allowed the detection of both MEP and silent-period parameters in the 500 ms following TMS. Stimulus intensity during testing was determined by adding intensity equal to 5% of the maximum stimulus output above the motor threshold. The mean of three consecutive trials was used to define the following parameters:

- Motor threshold (%), defined as the intensity required to elicit detectable MEPs with amplitudes of 0.05–0.15 mV in 50% of the stimuli, and expressed as the percentage of the stimulator's maximal output;
- MEP amplitude (mV), defined as the peak-to-peak amplitude between the largest negative and positive deflections following stimulus onset;
- Silent period duration (ms), measured from the MEP to the rebound of voluntary EMG activity. The EMG was recorded with

0.5 mV gain sensitivity, and the analysis time ranged from 200 to 500 ms.

2.5. Single-TMS: MEPs to single stimuli after a bimanual motor task

Motor cortex excitability was tested in three conditions: baseline, after exercise, and after rest. After each condition, three test pulses were presented at 2–3 s intervals (105% of motor threshold), during brief tonic contractions of the index finger on to the thumb. MEPs were measured from the first dorsal interosseous muscle of the non-dominant hand. During the baseline condition, subjects were tested after lying quietly and comfortably on a padded examination table for ≥30 min. In the exercise condition, subjects performed bilateral, repetitive opening and closing movements of the index finger toward the thumb with both digits extended (three or four movements per second). Three different exercise periods (lasting 30, 60, and 90 s respectively) were given to the subjects in progressive order. The inter-trial intervals between the different exercise blocks lasted about 1 min. In the rest condition, TMS pulses were delivered after subjects had lain quietly and comfortably on a padded examination table for 15 min without moving their fingers. The mean of the three MEPs obtained in each trial was used as the measure of MEP amplitude for the baseline period, immediately after each exercise period, and after the 15 min rest period. MEP amplitude of each exercise period and rest period was expressed as a percentage of the baseline MEP amplitudes. The subjects were instructed to perform the contraction of the target muscle with about 20% of their maximal force. We chose this level because TMS measures of post-exercise facilitation are similar when tested between 10% and 50% of the maximum force [29]. The ongoing EMG activity of the target muscle was monitored acoustically and visually on the oscilloscope. Subjects were given feedback if they were performing the motor task correctly.

2.6. Paired-pulse TMS: time-course of intracortical inhibition

MEPs were recorded through surface electrodes applied upon the opponent pollicis muscle, contralateral to the stimulated hemisphere, during complete relaxation of the target muscle. A focal butterfly-shaped coil was held tangential to the skull with the handle pointing backward at 45° lateral to the midline. Usually the "optimal" responses were elicited when the coil was placed 5–6 cm along the coronal line from Cz point (10–20 International System). A conditioning-test design was used to investigate the time-course of MEP inhibition. Paired stimuli were applied with conditioning pulses, delivered respectively 1, 2, 3, 4, 5, or 6 ms before test stimulation. The intensity of the conditioning pulse was maintained below the threshold (70% of the individual resting motor threshold for evoking responses in contracted muscles). Test pulses were delivered above threshold (120–110% of the individual resting motor threshold for eliciting relaxed MEPs). In each block, test and conditioning pulses at the different inter-stimulus intervals were randomly mixed. Several blocks of trials were performed in order to achieve a complete set of inter-stimulus intervals. Each block included 16 trials, eight having the test stimulus alone (unconditioned MEP) and eight having pairs of conditioning-test pulses delivered at one of the six inter-stimulus intervals (conditioned MEP). The sequence began and ended with the unconditioned trials, with the conditioned MEP trials in between. Mean amplitude of unconditioned and conditioned MEPs were calculated separately for each inter-stimulus interval. The amplitude of conditioned MEPs was expressed as the percentage of unconditioned MEPs' amplitude. The time-course was defined as the mean amplitude variation of conditioned MEPs (expressed as the percentage of unconditioned MEPs' amplitude) at each inter-stimulus interval.

2.7. Data analysis

2.7.1. Sleep macrostructure and microstructure

After verifying for their normal distribution, student's *t*-test for paired samples was used to compare the sleep data of the basal night vs the fragmented night.

2.7.2. Single-pulse TMS: MEPs, motor threshold, and silent period

Basal condition versus fragmented condition, regarding motor threshold, MEP amplitude, and silent period duration, and SSS, were measured using paired *t*-tests.

2.7.3. Single-pulse TMS: MEPs after a bimanual motor task

Single-sample *t*-tests were performed for each time-point in each condition, in order to determine whether MEP amplitudes were significantly different from baseline. Basal condition vs fragmented condition differences regarding MEP amplitude at the four time-points along the motor task (baseline, immediately after each of the exercises, and after rest) were evaluated using paired *t*-tests. One-way analysis of variance (ANOVA) was performed with repeated measures in order to evaluate the MEP amplitude changes along the motor task using the factors of conditions (pre-treatment versus post-treatment) and time (baseline, immediately after each of the exercises, and after rest).

2.7.4. Paired-pulse TMS: time-course of intracortical inhibition

Condition differences (basal condition vs fragmented condition) in the time-course of cortical inhibition were assessed using a 2 (condition) × inter-stimulus interval (1, 2, 3, 4, 5, 6 ms) repeated measure analysis of variance (ANOVA). To examine the condition × time-course interaction, separate ANOVA measures were performed for each condition using inter-stimulus interval as the within-condition factor. Condition differences (basal condition vs fragmented condition) at each inter-stimulus interval were measured using paired *t*-tests.

The assumption of sphericity was evaluated using the Mauchly test, and a Huynh–Feldt correction was used when necessary. When the Huynh–Feldt correction was applied, the original degrees of freedom and corrected *P*-values were reported. Post-hoc tests among the means were performed using paired *t*-tests, adjusted for the number of comparisons (Fisher's protected least-significant difference). Differences at $P \leq 0.05$ were considered significant.

3. Results

3.1. Sleep macrostructure and microstructure

No significant difference was found when comparing basal condition vs fragmented condition in total sleep time (430.3 ± 23.3 vs 428.9 ± 25.2 min respectively), or for sleep staging parameters or for sleep efficiency (96.4 ± 2. 8 vs 94.1 ± 4.5). Instead, SF determined a significant modification ($P \leq 0.05$) of the following microstructural data: total of cyclic alternating pattern (CAP) sequences, CAP rate, CAP rate of stage 2 of non-rapid eye movement (NREM) sleep, CAP rate of slow wave sleep, total number of A phases, number of A phases in stage 2 of NREM sleep, total number of A2 phases, and total number of A3 phases. SF determined a significant modification of the arousal index and the total number of arousals ($P < 0.001$ in both cases).

3.2. Single-pulse TMS: MEPs, motor threshold, silent period, and SSS

Comparisons between basal condition vs fragmented condition showed significant difference for motor threshold intensity (40.0 ± 4.4% vs 45.1 ± 4.9%, respectively) ($t = 2.75$, df = 6; $P = 0.03$).

Comparisons between basal condition vs fragmented condition showed no significant differences for MEP amplitude (9.9 ± 4.7 vs 9.3 ± 4.1 mV, respectively) ($t = 0.30$, df = 6; $P = 0.77$), or silent period duration (75.1 ± 14.7 vs 71.7 ± 21.5 ms, respectively) ($t = 0.40$, df = 6; $P = 0.70$).

Finally, we observed a significant increase in the SSS: 3.44 vs 4.00 ($P < 0.001$).

3.3. Single-pulse TMS: MEPs after a bimanual motor task

MEP amplitudes in basal condition vs fragmented condition at the four time-points in the bimanual task conditions are shown in Fig. 1.

Repeated ANOVA measures showed no significant effect of condition [$F(1, 12) = 0.23$; $P = 0.64$]. Repeated ANOVA measures showed a significant effect of time in each condition [basal condition $F(4, 34) = 2.83$; $P = 0.04$, fragmented condition $F(4, 34) = 3.12$; $P = 0.03$]. The t-tests comparing the two conditions at each time-point showed no significant differences in MEP. In both conditions, MEP amplitude was significantly larger than baseline immediately after the 30 s (basal condition $P = 0.01$, fragmented condition $P = 0.04$) and 60 s (basal condition $P = 0.008$, fragmented condition $P = 0.003$) time-periods, indicating the presence of post-exercise facilitation. There were no significant differences from baseline after the 90 s time (basal condition $P = 0.1$, fragmented condition $P = 0.3$).

In both conditions, MEP amplitudes after rest showed a significant increase in amplitude (basal condition $P = 0.01$, fragmented condition $P = 0.03$), indicating the presence of the delayed facilitation.

3.4. Paired-pulse TMS: time-course of intracortical inhibition

MEP amplitudes to test stimuli as a function of inter-stimulus interval for both conditions are shown in Fig. 2.

Comparisons between basal condition vs fragmented condition showed significant difference for motor threshold intensity (36.5 ± 4.9% vs 40.7 ± 501%, respectively) ($t = 2.99$, df = 6; $P = 0.02$). Repeated ANOVA measures showed no significant effect of condition [$F(1,12) = 2.98$; $P = 0.11$]. The t-tests comparing the two conditions at each inter-stimulus interval showed no significant differences. Repeated ANOVA measures showed a significant effect of inter-stimulus interval in each condition [basal condition $F(5,41) = 2.51$; $P = 0.05$, fragmented condition $F(5,41) = 7.50$; $P = 0.005$].

4. Discussion

The purpose of this study was to examine movement-related cortical plasticity and central motor inhibition in healthy good sleepers after a night of SF. These issues do not seem to have been addressed previously. The major finding was that there were no significant differences between the two conditions concerning the cortical inhibition/excitability and/or the cortical plasticity-related parameters. At first sight, this might seem a failure, but it is rather a significant step forward in understanding the complexity of sleep disorders.

4.1. MEP amplitude, motor threshold, and SSS

MEP amplitude was not different among conditions, confirming that the cortical–spinal function is not influenced by SF [30]. The fragmentation of sleep produces significant disruption of nocturnal sleep, reduces daytime alertness, and increases sleepiness [5]. Significant increases in the SSS and in the MT were observed in SF, which may be confirmatory features of this clinical behaviour [31,32]. Such features seem inconsistent with data from sleep-deprived patients, but there seems to be only a poor correlation

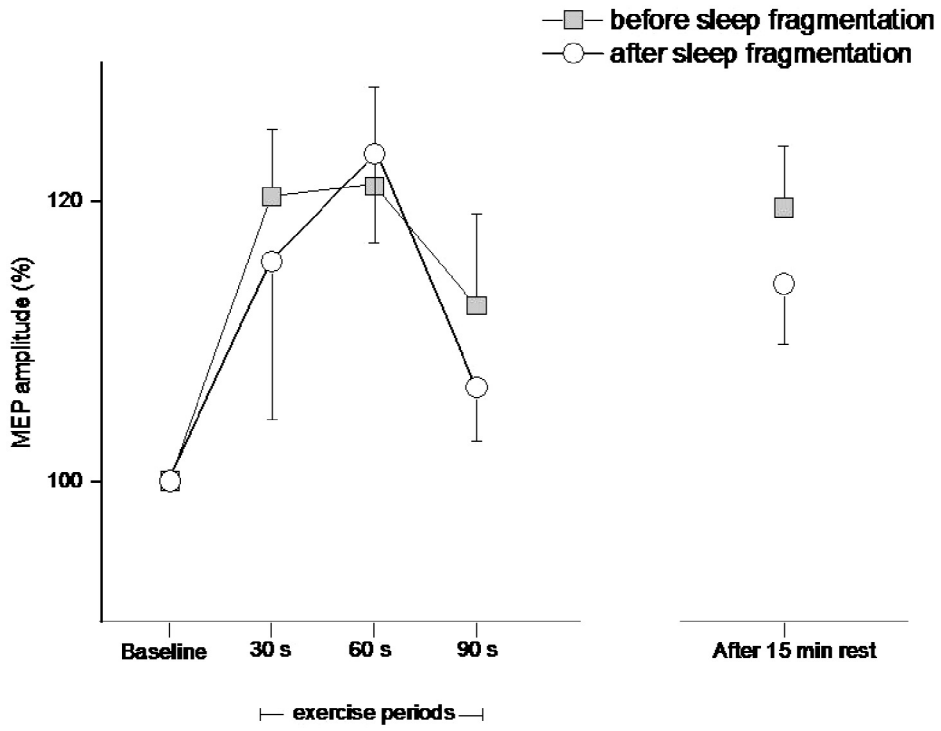

Fig. 1. Single-pulse transcranial magnetic stimulation: motor evoked potential (MEP) amplitude elicitated from the first dorsal interosseous from subjects tested at different times following a bimanual motor task. Note the significant increments of MEP amplitude after 30 and 60 s exercise periods (post-exercise facilitation) and after 15 min rest period (delayed facilitation) in both conditions.

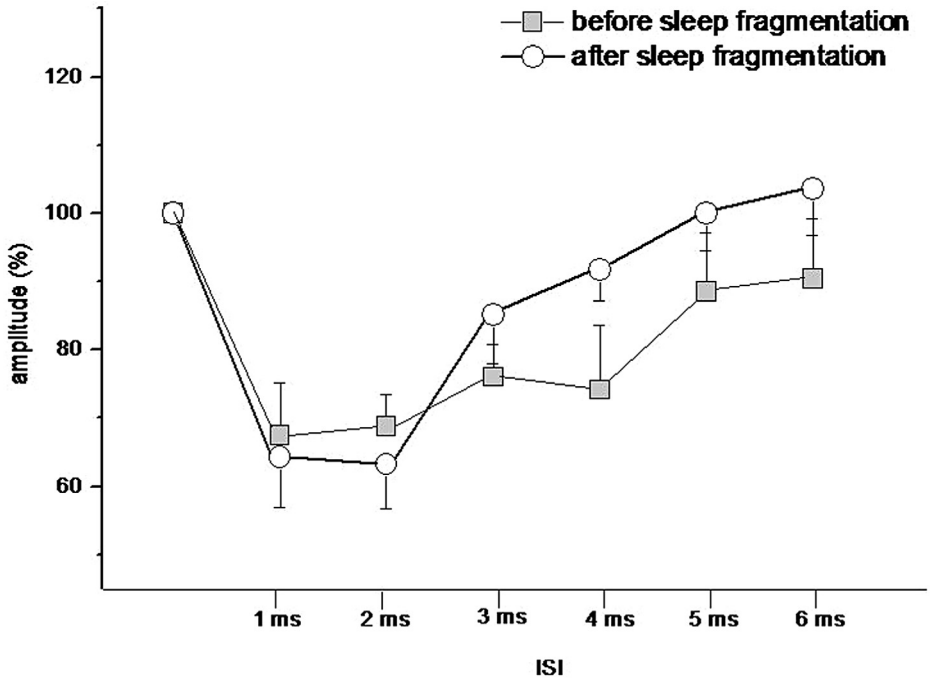

Fig. 2. Paired-pulse transcranial magnetic stimulation. The average time-course at different inter-stimulus intervals (ISI) (1–6 ms) in the different conditions. At each ISI, the size of the conditioned motor evoked potential (MEP) is expressed as a percentage of the size of the unconditioned MEP alone. Repeated analysis of variance measures showed a significant effect of the inter-stimulus interval in each condition.

between motor threshold and standard sleepiness scales in the measurement of subjective levels of sleepiness [12–16].

4.2. Central motor inhibition and silent period

After SF, the central motor inhibition and the silent period duration, normally considered indicators of inhibitory activity within primary motor cortex [14,18–20], showed no significant differences compared with the basal condition. By matching the present results with earlier ones of subjects submitted to different kinds of sleep loss [12–17] and in patients affected by different sleep diseases [18–20,33], it may be assumed that TMS results are very different in relation to the quality and the quantity of sleep. In our study, the sleep macrostructure was preserved so that the subjects had a satisfactory quantity of sleep and a good REM stage. On the contrary, in partial deprivation of REM sleep and in total SD, the sleep macrostructure and microstructure are supposed to be modified. We agree with Placidi et al., who emphasize the proconvulsant role of sleep loss, and suggest that REM SD could be the most effective sleep stage restriction in reducing the intracortical inhibitory processes [16,34].

4.3. Movement-related cortical plasticity

Sleep modifications could trigger changes in cortical excitability/inhibition, which consequently may influence sleep-dependent plasticity. Insufficient sleep may alter neuronal excitability and synaptic communication in neuronal networks involved in cognition, in motor learning, in memory, and in brain plasticity [35–38]. In our study, the sleep fragmentation did not affect movement-related cortical excitability; in fact, the post-exercise facilitation and the delayed facilitation were unchanged after the motor task. The mechanism for post-exercise facilitation is thought to be due to a transient increased excitability in the motor cortex [29,39]. The delayed facilitation seems to reflect an intracortical synaptic reorganization consequent to the performance of repetitive motor tasks [23,24]. The motor cortex is a highly modifiable structure and repeated practice or skill learning is associated with substantial representational plasticity [40]. During a motor task, the repeated activation of excitatory synapses in the central nervous system may possibly change the neural circuit dynamics and modify cortical excitability [41–46]. These phenomena, which may operate through the short-term and long-term potentiation (LTP) mechanisms, and/or the unmasking of pre-existing synapses on to motor cortex neurons, represent the neural substrates of physiological learning processes, important mechanisms for rapid (early) practice-dependent plasticity [45]. Consequently, the delayed facilitation we observed may be the functional evidence of intracortical synaptic reorganization, a main mechanism for motor skill learning. The persistence of post-exercise facilitation and delayed facilitation after SF suggests that this kind of sleep loss is insufficient to affect movement-related cortical plasticity in healthy good sleepers.

4.4. Sleep fragmentation and restless legs syndrome

Patients with RLS usually suffer from sleep disruption, and show markedly fragmented sleep, elevated SF index, and a significant abnormality in sleep microstructure [47–52]. Previously, in RLS patients we demonstrated alterations in both central motor inhibition and movement related cortical plasticity [18–20]. However, we did not find these alterations in subjects after SF. The model of experimental SF used in our study induces changes limited to the microstructure, leaving unchanged the sleep macrostructure; in this way we reproduced, in a laboratory, the effects of sleep disorders characterized by frequent and periodic disruptions of sleep, such as those caused by periodic limb movements in sleep (PLMS) in patients with RLS. Taken together, these data lead us to speculate that the alterations in cortical excitability found in RLS patients are intrinsically related to the underlying disease per se, rather than being directly associated with the SF present in the RLS. The theoretical reasoning behind this hypothesis is also supported by experimental pharmacological evidence. Dopamine-agonist treatments have

been shown to be effective in reducing PLMS index and subjective RLS severity [50,53], and, more important, are followed by normalization of the TMS alterations [20]. However, changes in sleep microstructure present in RLS do not seem to be affected by the treatment [52]. Since LTP-dependent processes such as practice-dependent plasticity are enhanced by dopamine [40], the classical "chicken and egg" dilemma seems to be solved: the TMS alteration we found in RLS patients may be a hallmark of this sleep disease and not a consequence of the poor quality of the sleep reported by these patients.

Conflicts of Interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2014.06.007.

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